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Assessment of Cadmium Exposure and
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American Vegetarian Population

Loma Linda Univ. Medical Center, CA

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

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ASSESSMENT OF CADMIUM EXPOSURE AND TOXICITY RISK
IN AN AMERICAN VEGETARIAN POPULATION

by

Rodney E. Willard, M.D.
Loma Linda University Medical Center
Clinical Laboratory
Loma Linda, California 92354

#R806006

Project Officer

Norman E. Kowal
Toxicology and Microbiology Division
Health Effects Research Laboratory
Cincinnati, Ohio 45268

HEALTH EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, NORTH CAROLINA 27711

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The complexities of environmental problems originate in the deep interdependent relationships between the various physical and biological segments of man's natural and social world. Solutions to these environmental problems require an integrated program of research and development using input from a number of disciplines. The Health Effects Research Laboratory, Research Triangle Park, NC and Cincinnati, OH, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. Wide ranges of pollutants known or suspected to cause health problems are studied. The research focuses on air pollutants, water pollutants, toxic substances, hazardous wastes, pesticides, and non-ionizing radiation. The laboratory participates in the development and revision of air and water quality criteria and health assessment documents on pollutants for which regulatory actions are being considered. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of environmental regulatory decisions involving the protection of the health and welfare of all U.S. inhabitants.

This report examines the possibility that vegetarians may have a higher cadmium exposure and accumulation than non-vegetarians.

F. Gordon Hueter, Ph.D.
Director
Health Effects Research Laboratory

ABSTRACT

It has been postulated that nonvegetarians may be exposed to less cadmium than vegetarians because of the cadmium-poor meat in their diet. This study attempts to test this possibility by measuring the cadmium exposure and accumulation in a population subgroup that includes many vegetarians. The conclusions are:

1. No statistical difference in cadmium exposure rate is demonstrated between the nonvegetarians and lacto-ovo-vegetarians of this study. 2. The cadmium exposure rate of the small group of pure vegetarians studied is in the direction anticipated (higher than nonvegetarians) but is not large enough to achieve statistical significance. 3. Cadmium accumulation in the critical organ (kidney) of subjects coming to autopsy is well below the range associated with renal injury. 4. Quantitative methods for measuring total urine protein are not as satisfactory for assessing tubular proteinuria as those developed to measure the small proteins that normally appear in the glomerular filtrate.

This report was submitted in fulfillment of Grant #R806006 by Loma Linda University Medical Center under the sponsorship of the U.S. Environmental Protection Agency. This report covers a period from August 1978 to August 1983.

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INTRODUCTION

Only 3% of the cadmium used in the United States is recycled. The remainder is dispersed into the biosphere where it may contaminate our drinking water and enter the food chain. Estimates base on autopsy data and environmental analysis have led to the fear that lifetime exposure and accumulation may be nearing a critical toxic level in the general population. Schroeder's animal experiments and several autopsy series have suggested a relation between unrecognized cadmium accumulation and hypertension. Up to 20% of the total body burden in smokers may be related to absorption from tobacco-born cadmium. Analysis of foods from cadmium-contaminated regions show variation in cadmium content. Cereals and other foods of plant origin contain higher concentrations than most foods of animal origin. Regardless of whether cadmium enters the body via the gastrointestinal tract from food and water or the respiratory tract via smoke or dust, the physiologic biochemical pathways result in its eventual binding to metallothionein. This 5000 dalton protein has the greatest binding affinity for cadmium of any protein studied thus far and easily carries the cadmium with it through the glomerular basement membrane into the renal tubular filtrate. The normal tubular function for conservation of amino acids and proteins results in the removal of cadmium-metallothionein complex from the filtrate into the tubular mucosa. The cadmium remains sequestered in the renal cortex for the rest of the life of the individual. When a sufficient concentration has accumulated, renal tubular dysfunction with cadmiumuria, beta-2-microglobuluria, and Fanconi syndrome become apparent. Additional accumulation leads to destruction of the renal cortex. The scarred contracted end stage kidney is indistinguishable from that of glomerulonephritis, pylonephritis, or hypertensive renal disease.

It has been postulated that nonvegetarians may be exposed to less cadmium than vegetarians because of the cadmium-poor meat in their diet. The food animal is exposed to the same water and plant sources as the human and the cadmium accumulates in his kidneys by similar physiologic mechanisms as in the human. As a consequence the skeletal muscle is depleted of its cadmium. Steak is cadmium-poor; sweet breads, especially kidneys and liver, are not. Food derived from skeletal muscle will dilute the cadmium obtained from the cadmium-accumulating food plants.

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This study attempts to test this possibility by measuring the cadmium exposure and accumulation in a population subgroup that includes many vegetarians. The study group is drawn from Southern California Seventh-day Adventists already enrolled in the ongoing Adventist Health Study. Seventh-day Advents are a small Protestant denomination with about three million members world-wide. Approximately 100,000 live in California. Church standards require that members abstain from the use of tobacco and alcoholic beverages. Previous studies have shown that over 98% of church members conform to these standards. The church also recommends other practices affecting life style that are not used as criteria for membership. A vegetarian diet is urged as the ideal. The use of unclean meats as biblically defined (pork, shellfish, etc.) is proscribed. Use of caffeine-containing beverages (coffee, tea, colas) and strong condiments (such as black pepper) are discouraged. Whole grains, nuts, vegetables and fruits as major dietary elements are recommended. A food industry specializing in protein products derived from plant sources has developed. Although these dietary practices have been taught for over 100 years, members vary widely in actual practice. A very few are pure vegetarians or vegans (PV). About half are lacto-ovo-vegetarians (LV). These use eggs and dairy products but abstain from meat, poultry and fish. The remaining non-vegetarians (NV) use clean meats and fish in their diet. About 17% drink one or more cups of coffee per day. Epidemiologic studies among this group began a quarter of a century ago in the context of unhealthful effects of tobacco usage. These led to the prospective Adventist Health Study begun in the 1970's. Approximately half of the California Adventist population are enrolled. They completed life style questionnaires and respond periodically to health status inquiries. Hospital record and death certificate information supplements health data obtained directly from the participants.

The public concern for identifying and minimizing environmental health risks mandates a monitoring of exposure rates. Following index population groups that have higher than average exposure to cadmium may be useful in providing warning of general population health risk.(1-6)

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CONCLUSIONS

1. No statistical difference in cadmium exposure rate is demonstrated between the nonvegetarians and lacto-ovo-vegetarians of this study.
2. The cadmium exposure rate of the small group of pure vegetarians studied is in the direction anticipated (higher than nonvegetarians) but is not large enough to achieve statistical significance.
3. Cadmium accumulation in the critical organ (kidney) of subjects coming to autopsy is well below the range associated with renal injury.
4. Quantitative methods for measuring total urine protein are not as satisfactory for assessing tubular proteinuria as those developed to measure the small proteins that normally appear in the glomerular filtrate.

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EXPERIMENTAL METHODOLOGY

Solicitation of Participants:

Live Subjects

From the computerized records of the Adventist Health Study, 50 pairs consisting of one SDA lacto-ovo-vegetarian and one SDA non-vegetarian were generated. The members of each pair were matched by age, sex, marital status, education, menopausal status, and occupational group. Since cadmium accumulates over the life span of the individual at a biological half life of approximately 20 years, these subjects were selected from the 40 to 60 year old age group. Another matching was attempted to develop a similiar paring with 50 pure vegetarians. Because these vegans are such a small fraction of those enrolled in the Adventist Health Study, the age requirements were relaxed to increase the possibility of recruiting a complete group. These subjects were identified only by a transformed identification number. The ID numbers of the subjects that matched were passed back to a computer routine that mailed letters explaining the study to subjects. Each subject was randomly assigned to one of the two dieticians for the rest of the study. The dietician was given a name, address, and phone number. Any further information the dietician received about the subject came directly from the subject. If a subject declined to participate, none of our staff learned anything further about the subject, even whether or not he was part of the Adventist Health Study.

Five to seven days after the introductory letter was mailed, the dietician called the participant. She briefly explained the study, answered any questions, and made an appointment for the first home visit. If the subject agreed to participate, a letter confirming the appointment and an introductory manual briefly explaining specimen collection was mailed. At the first interview a written consent was obtained and a detailed explanation of the collection procedure made both verbally and in the form of an extensive instruction manual.

When a subject declined to participate, a computer routine was used in an attempt to replace him. If he was a vegetarian

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subject the computer searched the files for another match. If this was unsuccessful, the dietician assigned to contact the matching subject was instructed to cancel the appointment for home visit with the matching subject, and expressed our regrets. If another match was found, and home visits with the original subject had not been scheduled, they were postponed until a few days after the new subject had received the introductory letter.

Once the first home visit had been completed, all subjects were carried through the completion of sample collection, regardless of withdrawals of matched subjects. The first visit was conducted by a research nutritionist. Any questions at this point were answered. Written consent was obtained from the study subject. Then the subject was asked to recall food intake in the previous 24 hours, using models and following a prod question sheet. The purpose of the unannounced recall was to determine if the subjects changed their diets while completing the three-day diary. Seventh-day Adventists in particular may have a number of ideas about an appropriate diet which they may not be following exactly. The knowledge that their diet is being analyzed may cause them to consciously or unconsciously change their eating.

A health history (exclusion criteria) questionnaire was administered by the nutritionist. This questionnaire included inquiries about a number of characteristics that were the basis for further exclusions. These included osteomas, major changes in diet in the last two years, and use of a number of drugs, including cancer chemotherapeutic agents and lipid lowering agents. The use of estrogens was recorded, but not used as grounds for exclusion.

The subject was instructed in the collection of urine and feces. A 2.5 liter container and a 1 (one) liter wide mouth plastic jar in an airline bag were provided for 24-hour urine collected on the second day of feces collection. This excluded the first voiding of that day and included the first voiding of the following day. The container provided contained a preservative (HCl). The fecal sample included every stool during three days. Plastic bags were provided to hang in the toilet, or a port-a-potty made available if so desired. After defecation the bag was removed, deposited in another plastic bag and the outer bag closed, and placed in a freezer chest packed with dry ice. This outer bag was labeled with the subject's identification number, the date, whether contaminated with urine, and a sequential number to identify the bowel movement. Subjects were instructed to urinate before defecating to minimize urinary contamination of the fecal sample. The plastic bag was hung in the toilet in such a way that there was some space in front of the bag for males to urinate. In case of females, a small amount of urine might fall into the bag. There also was space behind the bag to drop paper into the toilet. A small check list was used

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to identify any stools with urinary contamination. Employed subjects were provided with another freezer chest at their place of employment if they felt it likely they will defecate at work. This was labeled "Biological Materials for Scientific Research - Do Not Disturb or Remove". Our staff contacted the employer to deliver and pick-up the chest.

All subjects were asked to collect food samples.(7) All foods and beverages (except water) consumed were weighed on a gram scale. During the three days of fecal collection, the subject was asked to place about 2 ounces of food or beverage other than water, in a small plastic container labeled with the name of the food. Dressings, sauces, etc. added at the table were collected separately, as these may not be mixed homogenously on the plate with the foods with which they are eaten. A number of small containers, each containing 2 ounces of a different food could be placed in a larger container labeled for the date and the meal. The food samples were refrigerated but not frozen. Vitamin pills and other dietary supplements were collected in exact duplication of the amount used by the subject, and a record of the nutritional supplement's composition obtained by the dietician.

Each subject was given forms to record his complete dietary intake during the three days of fecal collection.(8) He was instructed to record all food and beverages consumed, and to save recipes of homemade foods.

The subject was given a day within the following next week to begin his dietary record and sample collection. Each collection period included one weekend day, that is it will be Thursday, Friday, and Saturday, or Sunday, Monday, and Tuesday.

The 24-hour recall was coded by the dietician for the dietary analysis the same day if possible.

On the second day of the collection period the same dietician called the subject to review the procedures and answer any questions. This allowed difficulties to be handled early. Specific questions were asked to ascertain comprehension and compliance.

On the workday following the sample collection (that is, a Monday or a Wednesday) the dietician retrieved the freezer chest containing the fecal and urine samples, another containing the food samples, and when used, a third containing samples from the subject's place of employment.

The dietician reviewed the three-day diary with the subject and edited the diary as necessary.

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The subjects height, weight, triceps skinfold, and blood pressure were measured.

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The dietician checked each food cup for sample adequacy and completeness and reviewed the urine and fecal samples collected for completeness.

The same day the dietary diary was coded whenever possible.

The dietary interviewer made an appointment for a venipuncturist to draw blood, and explain the 12-hour fast. This appointment was made as soon as possible after the period of food collection, within no more than seven days. It was not made during the food collection period, or on the day following, to prevent the fast from interrupting the subject's usual eating habits.

The venipuncturist visited the subject's home, usually early in the morning to draw the blood samples for the biochemical profiling. Those subjects living within easy driving distance from the medical center were scheduled to have the bloodwork drawn in the outpatient venipuncture station.

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Autopsy Subjects

Kidney, liver, pancreas and hair samples collected by cooperating pathologists in Southern California Seventh-day Adventist hospitals were obtained from subjects coming to autopsy. Life style and health data were obtained from hospital charts, next-of-kin, and from the Adventist Health Study data base.

Analytic Methods

Sample Preparation:

I. Stool Specimens

Frozen feces from the four-tailed collection bags were combined in a tared can lined with a cadmium free plastic bag and weighed. A equal mass of ultrapure deionized water was added and the mixture homogenized for 30 minutes on a shaker. A weighed amount of homogenate was dried to constant weight in a vacuum drying oven at 90 degrees Celsius. It was then wet digested using ultrapure nitric acid and hydrogen peroxide. An aliquot of the digestion mixture was diluted with ultrapure deionized water to the working range of the analytic method.

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II. Food Samples

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Each food serving aliquot was weighed, then combined with the other aliquots in proportion to its fraction of the total food ingested as reported in the subject's dietary diary. The total mixture was then homogenized in a Waring blender and aliquoted. A weighed amount of the food homogenate was then processed in the same manner as the fecal homogenate above.

III. Urine

The 24 hour collection was combined and its volume measured in a graduated cylinder. An aliquot was wet digested. The digestion mixture was further aliquoted and working standards added by the method of standard additions. These were mixed with a metal chelator, sodium diethyldithiocarbamate, and extracted into methyl isobutyl ketone to concentrate the cadmium into the working range of the analytic method.

Contamination Control

Ultrapure reagents for trace metal analysis were obtained. Ultrapure deionized water was used for all reagent preparation and final rinses of labware. All collection containers and devices, all storage containers, all processing containers, any material coming in direct contact with specimens were selected for low initial contamination, then soaked in ultrapure dilute nitric acid, and rinsed repeatedly with ultrapure deionized water. Any showing detectible amounts of cadmium were rejected. Sample processing and analysis was conducted in laboratory restricted to the project. Reagent blanks and eluates of containers were repeated on a regular basis to monitor for unsuspected contamination.

Cadmium Analysis:

Measurements were made in a Hitachi 170 Zeeman effect atomic absorption spectrophotometer using the 228.8 nm cadmium spectral line. 25 microliters of sample and standard mixture were introduced into the hollow carbon cuvette, dried at 120 degrees, ashed at 350 degrees and atomized at 2000 degrees Celsius. The method of standard additions was used to minimize matrix effects. Five replicate burns were made at each concentration of standard. Five different concentrations of standards spanning the working range of the instrument were used. The best least mean squares fit line through these data points was used to determine the cadmium content. National Bureau of Standards orchard leaves were processed and measured with each batch of sample determinations to assure instrumental and reagent performance.(9-14)

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Urine Protein

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Three quantitative urine protein methods were performed.

1. A reference method that employs an alcohol-phosphotungstic step to precipitate the protein. The washed precipitate is redissolved and quantitated using the biuret reaction. (15)
2. A traditional turbidometric method using trichloroacetic acid to precipitate the protein. (16)
3. A new stabilized dye-binding method using Coomassie Brilliant Blue G250 dye. (17)

The same human albumin standards and pooled controls were used for all three methods.

Urine Beta-2-Microglobulin

The B-2 microglobulin radio-immunoassay kit from Pharmacia used in previous international comparison studies was utilized. (19-21)

Biochemical Profile

The blood specimens were processed by the standard methods in routine use in the hospital clinical laboratory for patient care the same day they were obtained.

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SECTION 4

RESULTS

Live Subjects:

The following tables summarize the results of the biochemical profile measurements for non-vegetarians (NV), lacto-ovo-vegetarians (LV) and pure vegetarians (PV). The reporting units were those in routine use in the hospital laboratory at the time the measurements were performed and do not always conform to S.I. practice. The reference after each test name is to the method used.

Biochemical Characterization of Test Subjects

Constituent	NV	LV	PV
Serum Iron (22)			
mean (ug/dl)	93.3	87.4	107.3
range	28-148	27-181	52-194
n	49	48	18
Serum TIBC (22)			
mean (ug/dl)	311.4	314.7	320.2
range	235-405	249-429	252-499
n	49	46	18
Serum ferritin (23)			
mean (ng/ml)	126.0	61.9	67.4
range	5.5-404	2.7-177.7	20.3-166
n	50	49	18
Serum folate (24)			
mean (ng/ml)	10.57	11.9	14.3
range	1.4-31.8	3.2-38	4.1-34.2
n	50	49	18
Serum Vitamin B12 (25)			
mean (pg/ml)	696.3	681.0	683.2
range	309-3010	329-1314	297-1692
n	50	49	18

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Constituent	NV	LV	PV
Serum Hemoglobin (26)			
mean (gm/dl)	14.4	14.2	14.2
range	8.7-16.9	7.8-17.1	12.5-15.9
n	48	48	18
White Blood Cells (27)			
mean (k/ul)	5.97	5.23	4.92
range	3.7-10.9	2.6-7.4	2.7-8.3
n	48	48	18
Serum Calcium (28)			
mean (mmol/L)	2.4	2.4	2.4
range	2.2-2.8	1.6-2.6	2.2-2.5
n	49	50	17
Serum Total Protein (29)			
mean (gm/dl)	7.0	7.0	6.9
range	6.0-8.1	6.1-7.9	6.1-7.4
n	49	50	17
Serum Albumin (30)			
mean (gm/dl)	4.4	4.4	4.3
range	3.8-4.8	3.7-5.3	3.9-4.6
n	49	50	17
Serum Uric Acid (31)			
mean (mg/dl)	5.6	5.0	4.9
range	3.1-8.3	2.8-8.1	3.6-8.3
n	49	50	16
Serum Creatinine (32)			
mean (mg/dl)	1.0	1.0	1.0
range	0.7-1.4	0.6-1.4	0.4-1.3
n	49	50	17
Serum Urea Nitrogen (33)			
mean (mg N/dl)	15.3	14.5	13.6
range	7-25	5-23	8-23
n	49	50	17
Serum Sodium (34)			
mean (mmole/liter)	140.6	138.7	140.8
range	125-147	108-145	131-146
n	49	49	17
Serum Potassium (34)			
mean (mmole/liter)	4.2	4.3	4.1
range	3.1-5.3	2.8-5.4	3.2-5.4
n	46	42	17

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Constituent	NV	LV	PV
Serum Bicarbonate (35)			
mean (mmole/liter)	24.7	24.6	25.4
range	19.0-30.0	21.0-28.0	19-30
n	49	48	17
Serum Chloride (35)			
mean (mmole/liter)	105.5	104.9	104.7
range	98-111	86-111	97-110
n	49	49	17
Serum Phosphorus (36)			
mean (mmole/liter)	1.03	0.98	0.95
range	0.6-1.5	0.6-1.3	0.7-1.3
n	49	50	17
Fasting Plasma Glucose (37)			
mean (mg/dl)	89.6	86.7	84.0
range	34-299	31-154	61-127
n	49	47	17
Serum Triglyceride (38)			
mean (mg/dl)	119.9	120.6	117.3
range	47-353	40-580	43-346
n	49	50	17
Serum Cholesterol (39)			
mean (mg/dl)	211.3	192.5	172.4
range	140-310	128-322	131-238
n	49	50	17
Serum Total Bilirubin (40)			
mean (mg/dl)	0.69	0.64	0.69
range	0.2-1.5	0.2-1.7	0.4-1.0
n	49	50	17
Serum Direct Bilirubin (40)			
mean (mg/dl)	0.12	0.12	0.11
range	0.1-0.3	0.1-0.3	0.1-0.2
n	49	45	17
Serum Lactate Dehydrogenase (LDH) (41)			
mean (U/L)	186.4	197.8	216.9
range	139-254	84-466	161-449
n	49	50	17
Serum Aminoaspartate Transaminase (SGOT) (42)			
mean (U/L)	19.7	21.19	22.5
range	10-37	8-39	14-60
n	49	50	17

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Constituent	NV	LV	PV
Serum Aminopyruvate Transaminase (SGPT) (42)			
mean (U/L)	20.5	17.9	18.1
range	8-44	7-40	8-51
n	49	49	17
Serum Alkaline Phosphatase (43)			
mean (U/L)	61.9	66.3	75.6
range	34-103	35-173	41-153
n	49	49	17
Urine Protein (44)			
mean (mg/day)	144.8	154.0	52.6
range	0-756	0-796	0-197
n	50	50	18
Urine Beta-2-Microglobulin (19)			
mean (ng/day) ^{1/2"}	79.9	68.8	223
range	2.7-700	3.8-190	9.9-2544
n	50	50	18
Urine Creatinine (32)			
mean (mg/day)	1340	1207	1006
range	623-2496	158-2921	393-1709
n	50	50	18

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The following tables summarize the cadmium measurements done on the samples from the live subjects. The results are reported in micrograms Cadmium per day for non-vegetarians(NV), lacto-ovo-vegetarians(LV) and pure vegetarians(PV).

Food Cadmium Ingestion
ug Cd/day

	NV	LV	PV
mean	29.3	31.6	37.4
range	9.3-63.0	10.0-73.5	13.8-87.5
n	36	39	15

Stool Cadmium Excretion
ug Cd/day

	NV	LV	PV
mean	23.7	25.5	35.1
range	7.6-74.4	5.2-65.5	9.2-55.2
n	42	46	17

Urine Cadmium Excretion
ug Cd/day

	NV	LV	PV
mean	0.88	0.76	0.73
range	0.12-2.9	0.087-5.1	0.12-1.48
n	48	46	16

Statistical analysis by Student's t test for fails to confirm a significant difference between groups and between matched pairs.

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Autopsy Subjects:

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Results of the tissue cadmium concentration measurements are reported as micrograms Cd per gram wet tissue.

Autopsy Tissue Cadmium Concentration

Tissue	NV	LV
kidney		
mean	16.8	17.3
range	6.5-37.1	2.2-48.4
n	9	17
pancreas		
mean	1.32	1.09
range	0.07-4.7	0.04-3.8
n	10	16
liver		
mean	1.88	2.67
range	0.4-4.7	1.2-6.8
n	8	16
hair		
mean	0.3	0.45
range	0.12-4.08	0.23-1.01
n	2	5

Data analysis using Student's t test for unequal size groups fails to show statistically significant differences.

Flour:

Hard winter wheat flour used in the commercial manufacture of meat analogs was obtained from four milling companies that supply manufacturers of meat analogs sold in southern California. Similar flour was obtained from two health food stores that cater to those making their own meat analogs at home.

1. Miller G	11.3 ng/gram of flour
2. Miller H	66.4 ng/gram
3. Miller I	100. ng/gram
4. Miller M	77.3 ng/gram
5. Market S	32.2 ng/gram
6. Market L	319. ng/gram

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An interinstitutional comparison of accuracy of the urine cadmium measurements was made by split sample analysis. Each of ten pooled urine samples were divided into two aliquots. One group of samples was sent for analysis to the Swedish Hospital Medical Center in Seattle, Washington, and the other analyzed in our laboratory. Specimens 6 & 9 were identical as were 7 & 10.

URINE CADMIUM Split Sample Analysis

I.D. #	LLUMC	SHMC
1	26.8 ng/dl	25 ng/dl
2	118	120
3	152	126
4	294	268
5	123	121
6	78.0	83
7	83.4	86
8	12.0	69
9	85.0	83
10	51.2	65

Linear regression analysis between results of the two institutions on each pair gives a correlation coefficient of 0.970, a y intercept of 24 and a slope of 0.79.

Comparison of Methods for showing Proteinuria

Method	Range	Units	Mean	S.D.	n
Biuret (15)	0-796	mg/day	133.8	162.8	118
TCA (16)	0-235	mg/day	25.5	44.5	117
Coom.Blue (17)	1.23-287	mg/day	61.5	53.1	117
B-2-M (19)	1.99-2544	ng/day	94.5	241.0	118

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SECTION 5

DISCUSSION

Anticipated differences in cadmium exposure rates between vegetarians and nonvegetarians is dependent upon the replacement of cadmium-rich calories from cadmium accumulating foods by cadmium-poor calories. For this difference to be apparent, the food of the vegetarian must contain more cadmium than that of the nonvegetarian. The ideal situation for demonstrating the difference is in the case of a single major food source for each group. This was the case for the Japanese eating rice grown in cadmium-contaminated water. But for the situation where there are a variety of foods, the differences may be masked. The non-vegetarian may be getting higher than background exposure by including liver, kidney and shell fish in his diet. The vegetarian who obtains the major portion of his food from the national food distribution system averages his exposure by the mixing of foods grown in cadmium-poor districts with those of contaminated areas. Another difficulty in demonstrating differences is in the classification of the subjects. In the strictest sense there are no pure vegetarians unless they grow all the food they eat. Process foods usually include milk or egg solids and other nonvegetable additives. And anyone depending upon the prepared food industry is getting more than he realizes. Self assignment to pure vegetarian, lacto-ovo-vegetarian or nonvegetarian groups may represent the person's ideal for his dietary practice, rather than his invariant practice. Of those pure vegetarians in the Adventist Health Study we contacted, most use some dairy products occasionally. Even the nonvegetarian group may be subject to cultural influence from its Adventist background to eat less meat than the general population does.

The lack of difference between the nonvegetarian group and the lacto-ovo-vegetarians group in this study is probably due to several factors. In spite of attempts to control confounding factors by matching subjects by age, sex, occupational group, etc., there still remain many differences - genetic, geographic origins, past changes in life style - to mask diet-induced differences. Both groups are subject to environmental contamination from tobacco smoke and other erratic sources. Both replace a portion of the higher cadmium content plant foods in their diet by cadmium-poor foods, dairy products for the vegetarians and skeletal muscle meat cuts for the nonvegetarians. It is unfortunate that we could not recruit a larger group of pure vegetarians to reduce the dietary overlap.

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The subjects in the autopsy study are not easily categorized into well-defined dietary groups. A few were life-long lacto-ovo-vegetarians but many became Adventists later in life and had made significant life style and dietary changes. Several had been heavy cigarette smokers. Thus the probable rate of exposure had varied significantly at different times of their lives. Even so the total renal cortex accumulation remained below the risk threshold of 200 micrograms cadmium per gram of wet tissue.

The concentration of cadmium in flour used in making meat analogues is in the range reported by other studies. The highest value was from a sample obtained from a partially open flour bin in a market where it may have been contaminated by the environment.

Urine cadmium concentration was at the low end of the working curve of the instrument. Its measurement was facilitated by the concentration achieved by the extraction step.

Urine protein methods are tuned to measure albumin. In the typical clinical setting, the physician is most interested in proteinuria as an indicator of glomerular dysfunction. Since the major protein appearing in the urine as a consequence of glomerular injury is albumin, such methods meet the clinical need. They vary widely in their response to other proteins. The smaller proteins normally pass through the glomerular basement membrane but are removed from the glomerular filtrate by the tubular epithelium. In the setting of tubular dysfunction or injury these small proteins replace albumin as the constituents of greatest clinical interest. But most methods grossly underestimate or fail to detect them. In our study this methodological shortcoming is evidenced by the lack of correlation between beta-2-microglobulin excretion and total protein excretion. Tubular proteinuria must be looked for specifically by urine protein electrophoresis or by specific assay of one or more of the small protein species.

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APPENDIX I

Project Staff:

Rodney E. Willard, M.D., Principal Investigator

Roland C Phillips, M.D., D.P.H., Adventist Health Study director

Beverly Calkins, D.H.S., Digestive Physiology Study coordinator

Ronald H. Hillock, Ph.D., clinical chemist

James Blankinship, Ph.D., nutritionist

Jan Kuzma, Ph.D., biostatistician

Milton C. Crane, M.D., endocrinologist

Betty Kettering, dietician

Donna Whitaker, dietician

Rosalind Phillips, dietician

Keith Hurinouchi, food technologist

Suzanne R. Hebert, clinical chemist

Wesley Ferrari, research assistant

William Winsor, research assistant

Michall Malotte, research assistant

Judy Van Arsdale, research assistant

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Appendix II

Instruction Sheet for Test Subjects

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DIRECTIONS FOR COLLECTING FOOD PORTIONS

1. Each time you eat or drink something (except water) fill one of the provided containers with the same food or liquid. Place each item in a different container (for example, mashed potatoes in one container; gravy in another container). You need not save more than 1/2 of small portions of foods such as margarine, butter, peanut butter, or jam. Save 2 containers full if you eat or drink 2 cups or more of anything. Be sure to FILL each container, and to save one portion for each serving you eat or drink.
2. Close the lid securely.
3. On the label on the top of the container, write the name of the item, the day number (1, 2, or 3), and the food number from the Food Diary sheet.
4. Record each item in your Food Diary.
5. Store the containers in your refrigerator in the tray provided for that time period.

BE SURE THAT YOU ---

- A. Collect representative samples (not from the edge, crust only, etc.)
- B. Use separate containers if foods are served separately. Examples: sauces, gravies, spreads, salad dressings, cream, jam, syrup, taco ingredients, self made salad ingredients, submarine sandwich ingredients
- C. Use a single container if foods are served already well mixed. Examples: stews, casseroles, potato salad, carrot raisin salad, cocktail
- D. Cut a representative cross-section portion from the center of solid foods. Examples: simple sandwiches, meat substitutes, roasts
- E. After eating out, keep the samples as cool as practical under the circumstances. Place in food compartment of of flight bag. Store the samples in refrigerator.

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STOOL SAMPLE COLLECTION DIRECTIONS

1. Save ALL stools during the three-day period.
2. Keep the following supplies near the commode:
Covered chest containing dry ice
Plastic bags with long tabs
Seal-sealing plastic bags with labels
Tape
Pen
"Stool sample checklist"
3. For each bowel movement, follow this method:
 - a. Before the bowel movement, open one plastic bag with long tabs.
 - b. Using the tape provided, tape the bag to the outside of toilet bowl.
 - c. Using the glove, drop a chip of dry ice from the small chest into the bag.
 - d. During the bowel movement, make sure that the entire stool falls into the plastic bag.
 - e. Nothing else, such as urine or toilet tissue should get into the plastic bag.
 - f. Wash your hands.
 - g. Open the next self-sealing bag and make sure it has the correct number in the sequence.
 - h. Remove the bag containing the stool from the commode.
 - i. Tie the tabs of the first bag and place it into the self-sealing bag.
 - j. Seal the outer bag.
 - k. Quickly place the sealed sample into the large dry ice chest.
 - l. Replace the foam and cardboard lids tightly and fasten the strap.
 - m. Wash your hands.
 - n. Fill in the "Stool Sample Checklist."
4. Caution: Dry ice can "burn" skin. Use gloves, not fingers.
5. Keep urine, toilet tissue, and anything else out of stool samples.
6. If the dry ice shrinks down to the "Refill Line", call (collect, if necessary) to one of the following numbers:
824-1921
796-7311, extension 3707

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STOOL SAMPLE CHECKLIST

Directions: On the blank, write the number of the day on which the sample was collected. Circle Yes or No whether it came in contact with any urine.

Sample Number	Day Number (1,2, or3)	Time	Contact with Urine (Circle yes or no)	
			Yes	No
1.				
2.	6-1/2"			
3.				
4.	9-1/8"			
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24-HOUR URINE SAMPLE COLLECTION DIRECTIONS

1. When you get up in the morning, void the first urine - discard it. Start collection with the next urination. Urinate directly into the container.
2. Write the time and the date on the label on the container.
3. During the day and night, save all urine, using the container.
4. Keep the containers of urine in a cool place (not freezer) out of sunlight.
5. End the collection the next morning after collecting the first urination.

MAIN POINTS:

1. Save ALL urine
2. Use provided containers
3. Keep it cool
4. Keep it out of sunlight
5. Keep it clean

AWAY FROM HOME: Carry the container with you in this handy flight bag so that you will not lose any of the 24-hour amount. Place in a cool place when you return home.

FORGOTTEN CONTAINER: Use any washed clean container. Cover. Refrigerate, or get it to the container with preservative within one hour (or within 30 minutes if 80 or higher.)

KEEP CLEAN: Void before moving bowels. Do not drop toilet tissue into container. Use provided containers.

PREVENT SPILLS: Keep in level, protected place, away from pets, or children. Do not handle when hands or outside of container are wet or slippery.

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LOST URINE: If any urine is lost, start collection over again the next morning in one of the provided containers that has not yet been used. If you do not have two containers left, call the following numbers: 796-7311, extension 3707 or 824-1921.

CAUTION: PRESERVATIVE CAN CAUSE BURNS. Rinse thoroughly with clear water if preservative contacts skin, clothing, or floor.

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EPA-287 (Cir.)
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Live Subjects - Cadmium Ingested from Food

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I.D.	ug Cd/d	ug Cd/d	diet category
001-04		22.5	LV
002-02	21.3		NV
002-18		38.7	LV
010-02	32.1		NV
010-03		29.6	LV
016-01	16.6		NV
016-02		15.0	LV
017-02		32.6	LV
017-03	11.3		NV
020-01		73.5	LV
020-04	25.4		NV
021-02		64.3	LV
021-07	31.6		NV
022-01		26.9	LV
022-02	52.7		NV
028-01		25.3	LV
028-12	47.3		NV
029-01		34.2	LV
029-04	26.4		NV
032-01	41.2		NV
032-02		24.0	LV
033-01	16.4		NV
033-14		9.9	LV
034-07		24.0	LV
034-14	19.6		NV
036-01		24.9	LV
036-04	51.2		NV
040-02		34.5	LV
040-13	32.2		NV
042-02	20.9		NV
042-03		64.8	LV
043-03		24.0	LV
043-10	63.3		NV
044-02		35.1	LV
044-11	35.6		NV
049-01		43.8	LV
049-04	15.4		NV
052-01		19.9	LV
052-02	58.1		NV
060-01	48.5		NV
060-02		59.9	LV
064-12	27.4		NV
064-21		33.1	LV
066-07		31.4	LV
066-08	27.2		NV
075-02		22.2	LV
075-11	11.2		NV

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I.D.

ug Cd/d

ug Cd/d

diet category

078-02	22.6		NV
078-09		47.2	LV
081-01	9.3		NV
081-10		44.2	LV
083-01	42.0		NV
083-02		41.3	LV
091-03		26.1	LV
093-01	27.0		NV
093-02		51.4	LV
098-02	32.2		NV
098-03		13.4	LV
102-04		11.3	LV
102-07	17.9		NV
109-02	14.4		NV
109-19		28.7	LV
112-02		25.7	LV
112-21	24.0		NV
114-02	25.0		NV
114-07		16.6	LV
116-03	22.8		NV
116-04		21.6	LV
118-04		34.0	LV
119-02		23.3	LV
119-15	46.1		NV
120-09	17.9		NV
120-12		14.8	LV
121-01	21.6		NV
121-08		18.1	LV
501-02		29.6	PV
505-51		19.8	PV
514-09		35.1	PV
515-10		47.0	PV
516-11		46.6	PV
517-12		19.6	PV
520-14		55.8	PV
544-23		87.5	PV
546-24		23.6	PV
555-30		38.6	PV
562-33		29.4	PV
564-34		31.8	PV
581-43		35.6	PV
583-44		13.8	PV
591-47		47.1	PV

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I.D.	Stool Cadmium Excretion:		diet category
	ug Cd/d	ug Cd/d	
001-04		8.8	LV
002-18		16.3	LV
010-02	34.0		NV
010-03		19.5	LV
016-01	47.1		NV
016-02		35.2	LV
017-02		10.4	LV
017-03	27.3		NV
020-01		40.7	LV
020-04	74.4		NV
021-07	19.4		NV
022-01		52.0	LV
022-02	36.2		NV
027-02		36.0	LV
027-23	25.4		NV
028-01		60.4	LV
028-12	14.4		NV
029-01		19.7	LV
029-04	20.3		NV
032-01	13.3		NV
032-02		27.9	LV
033-01	18.9		NV
033-14		22.0	LV
034-07		14.8	LV
034-14	27.8		NV
036-01		50.9	LV
036-04	26.7		NV
037-02		19.0	LV
037-17	17.3		NV
040-02		65.6	LV
040-13	25.6		NV
042-02	8.7		NV
042-03		5.2	LV
043-03		16.2	LV
043-10	7.9		NV
044-02		47.1	LV
044-11	26.7		NV
049-01		25.3	LV
049-04	16.1		NV
052-01		24.6	LV
052-02	19.2		NV
054-01	34.7		NV
054-02		14.4	LV
060-01	27.9		NV
060-02		56.7	LV
064-12	15.7		NV
064-21		35.3	LV
066-07		12.6	LV
066-08	10.7		NV

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I.D.	ug Cd/d	ug Cd/d	diet category
075-02		30.2	LV
075-11	48.3		NV
078-02	8.5		NV
078-09		17.3	LV
081-01	17.6		NV
081-10		16.0	LV
083-01	30.9		NV
083-02		33.9	LV
091-03		14.3	LV
091-14	24.5		NV
093-01	17.7		NV
093-02		26.7	LV
098-02	16.9		NV
098-03		18.3	LV
102-04		25.8	LV
102-07	16.3		NV
103-08	23.3		NV
103-13		22.1	LV
104-04		5.8	LV
107-01	7.7		NV
109-02	22.8		NV
109-19		20.4	LV
110-02	24.3		NV
111-09		19.3	LV
112-02		15.5	LV
112-21	41.2		NV
113-01		34.7	LV
114-07		14.5	LV
116-03	25.7		NV
116-04		14.5	LV
118-01	23.0		NV
118-04		13.3	LV
119-02		15.0	LV
120-09	16.5		NV
120-12		14.0	LV
121-01	18.5		NV
121-08		28.1	LV
122-03	21.7		NV
123-08		40.2	LV
501-02		42.9	PV
503-03		23.6	PV
505-51		11.7	PV
514-09		13.1	PV
515-10		48.0	PV
516-11		53.7	PV
517-12		25.0	PV
520-14		55.3	PV

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I.D.

ug Cd/d

ug Cd/d

diet category

544-23
546-24
551-27
555-30
562-33
564-34
581-43
583-44
591-47

48.0
34.5
28.1
48.7
39.5
33.0
9.3
53.8
31.4

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Urine Cadmium Excretion Data:

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Nonvegetarians

I.D.	mL/d	ug Cd/d	ug Cd/L
002-02	1882	1.17	0.62
016-02	536	0.17	0.32
017-03	861	0.18	0.21
020-04	1221	0.60	0.49
021-07	946	1.99	2.10
022-02	1356	0.52	0.39
027-23	906	0.47	0.51
028-12	2203	0.96	0.44
029-04	1152	1.11	0.96
032-01	1806	0.87	0.48
033-01	1561	0.35	0.23
034-14	1697	1.26	0.74
036-04	1938	0.15	0.07
037-17	1815	0.33	0.18
040-13	2708	2.46	0.91
042-02	2342	2.90	1.24
043-10	1082	0.20	0.18
044-11	1081	1.62	1.50
049-04	1078	1.09	1.01
052-02	1899	1.67	0.88
054-01	1264	1.14	0.90
060-01	730	1.93	2.65
064-12	1527	0.45	0.29
066-08	2137	0.21	0.10
075-11	1911	1.67	0.87
078-02	872	0.12	0.14
081-01	1966	0.96	0.49
083-01	609	0.42	0.69
091-14	842	1.22	1.45
093-01	1802	1.33	0.74
098-02	2247	0.98	0.44
102-07	1083	0.74	0.69
103-08	1453	0.87	0.60
104-11	1498	0.40	0.27
107-01	1775	0.91	0.51
109-02	1963	0.75	0.38
110-02	869	0.44	0.50
111-16	2049	1.38	0.67
112-21	1020	0.91	0.90
113-16	537	0.15	0.28
114-02	869	0.86	0.99
116-03	1380	0.64	0.46
118-01	907	0.69	0.76
119-15	1978	0.24	0.12

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I.D.	mL/d	ug Cd/d	ug Cd/L
120-09	1611	0.48	0.30
121-01	2515	1.47	0.58
122-03	1573	0.65	0.41
123-17	1010	0.38	0.38

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Lacto-ovo-vegetarians

I.D.	mL/d	ug Cd/d	ug Cd/L
001-04	1546	0.99	0.64
002-18	1005	0.47	0.47
017-02	1965	1.32	0.67
020-01	1264	1.00	0.79
021-02	1768	0.77	0.44
022-01	1261	0.15	0.12
027-02	587	0.53	0.90
028-01	1196	0.95	0.80
029-01	2812	5.10	1.81
033-14	2487	0.09	0.04
034-07	1457	0.95	0.65
036-01	1457	0.71	0.49
037-02	1368	0.87	0.64
040-02	2518	0.21	0.08
042-03	664	0.89	1.34
043-03	481	0.17	0.34
044-02	1002	0.39	0.39
049-01	1609	0.10	0.06
052-01	2655	0.09	0.03
054-02	1023	0.55	0.54
060-02	2896	1.63	0.56
064-21	1202	0.35	0.29
066-07	1703	0.20	0.11
075-02	781	1.04	1.33
078-09	1934	0.60	0.31
081-10	1784	0.46	0.26
083-02	390	0.54	1.38
091-03	1819	0.83	0.46
093-02	940	0.39	0.41
098-03	2250	0.58	0.26
102-04	1450	0.19	0.13
103-13	1152	0.44	0.38
104-04	1559	1.01	0.65
107-12	1161	0.24	0.21
109-19	2121	0.76	0.36
111-09	1709	0.69	0.40
112-02	1859	1.69	0.91
113-01	438	0.10	0.22
114-07	834	0.19	0.23
116-04	794	0.33	0.41
118-04	1682	0.74	0.44
119-02	1077	0.74	0.68
120-12	2277	0.55	0.24
121-08	1579	2.72	1.72
122-22	1103	1.02	0.92
123-08	1280	0.56	0.43

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Pure Vegetarians

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I.D.	mL/d	ug Cd/d	ug Cd/L
501-02	2186	1.34	0.61
503-03	1118	0.92	0.82
505-51	1987	0.50	0.25
514-09	2168	1.48	0.68
515-10	1183	0.39	0.33
516-11	2997	1.13	0.38
520-14	2471	0.12	0.05
544-23	1419	0.62	0.44
551-27	1845	1.42	0.77
555-30	1820	0.27	0.15
562-33	1153	0.45	0.39
564-34	1924	0.49	0.25
576-40	1692	0.47	0.28
581-43	1191	1.31	1.10
583-44	1248	0.37	0.30
591-47	2677	0.44	0.17

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Autopsy Kidney Cadmium Concentration:

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I.D.	ug Cd/g	Diet Category
13-19-07	22.9	NV
05-99-98	7.1	NV
25-58-36	15.3	NV
50-78-90	6.5	NV
52-25-64	7.3	NV
27-50-28	11.4	NV
41-26-85	10.6	NV
36-18-24	32.6	NV
19-25-93	37.1	NV
47-94-87	13.3	LV
22-19-49	6.3	LV
12-48-09	12.9	LV
01-00-49	31.7	LV
13-44-90	2.2	LV
48-08-64	21.5	LV
01-85-63	21.2	LV
15-49-51	18.7	LV
04-97-84	6.8	LV
07-07-98	3.0	LV
09-92-89	48.4	LV
43-81-00	18.0	LV
32-98-29	18.2	LV
37-75-23	12.6	LV
02-77-46	19.6	LV
12-04-03	35.4	LV
23-26-07	4.4	LV

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Autopsy Pancreas Cadmium Concentration:

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I.D.	ug Cd/g	Diet Category
13-19-07	4.67	NV
05-99-98	0.63	NV
25-58-36	0.98	NV
19-25-93	2.42	NV
50-78-90	1.58	NV
52-25-64	0.07	NV
27-50-28	0.48	NV
41-26-85	0.45	NV
14-20-14	0.31	NV
36-18-24	1.62	NV
01-00-49	3.78	LV
36-39-02	1.93	LV
02-77-46	0.30	LV
09-92-89	1.91	LV
13-44-90	0.61	LV
22-19-49	1.10	LV
12-48-09	0.76	LV
48-08-64	0.70	LV
01-85-63	0.08	LV
15-49-51	1.43	LV
04-97-84	1.47	LV
07-07-98	0.98	LV
37-75-23	0.76	LV
32-98-29	0.04	LV
12-04-03	1.00	LV
43-81-00	0.54	LV

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Autopsy Liver Cadmium Concentration:

I.D.	Cd/g	Diet Category
19-25-93	4.71	NV
05-99-98	1.67	NV
25-58-36	0.96	NV
50-78-90	1.93	NV
52-25-64	0.41	NV
27-50-28	0.40	NV
41-26-85	0.39	NV
36-18-24	4.60	NV
12-48-09	1.37	LV
01-00-49	3.91	LV
47-94-87	5.14	LV
22-19-49	3.04	LV
13-44-90	3.10	LV
48-08-64	2.32	LV
01-85-63	1.22	LV
15-49-51	1.01	LV
04-97-84	1.54	LV
07-07-98	1.28	LV
46-42-75	1.92	LV
09-92-89	2.10	LV
43-81-00	6.85	LV
37-75-23	1.12	LV
02-77-46	3.44	LV
12-04-03	3.38	LV

Autopsy Hair Cadmium Concentration:

I.D.	ug Cd/g	Diet
25-58-36	0.12	NV
14-20-14	0.41	NV
12-48-09	0.25	LV
37-75-23	0.29	LV
32-98-29	0.47	LV
22-19-49	0.23	LV
12-04-03	1.00	LV

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Autopsy Clinical Data:

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I.D.	Age	Sex	B.P.	Diet	Diagnosis
13-19-07	49	m	120/80	NV	chronic lymphocytic leukemia
47-94-87	83	m	120/70	LV	acute postlateral MI, parotid CA with lung metastases
22-19-49	74	m	178/100	LV	brain stem hemorrhage
05-99-98	68	f	110/75	NV	acute and old MI
12-48-09	70	m	120/70	LV	adenoca of colon with metastases
25-58-36	69	m	156/88	NV	nodular lymphocytic lymphoma
01-00-49	83	f	120/80	LV	mesothelial carcinomatosis
19-25-93	83	f	130/70	NV	smoked 2 packs/d multiple brain abscesses, peptic ulcers x 20 yrs
13-44-90	70	f	120/70	LV	aspiration pneumonia, chronic renal tubular acidosis
48-08-64	61	m	120/70	LV	cerebral astrocytoma, pulmonary embolus
01-85-63	68	m	160/90	LV	Ruptured sac. aneurysm of basilar artery, diabetes mellitus
15-49-51	32	m	102/80	LV	primary pulmonary hypertension with hemorrhagic cor pulmonale
04-97-84	79	f	120/80	LV	adenoca of cecum with metastases
07-07-98	77	f	114/70	LV	acute MI
46-42-75	71	f	150/68	LV	Peritonitis with perforation, Ca of cervix post radiation
37-75-23	83	m	170/90	LV	massive cerebral hemorrhage
36-39-02	39	f	170/100	LV	smoked 1 pack/d, SLE, Bronchopneumonia,
50-78-90	72	m	110/90	NV	Hypertensive Heart Disease ca of lung with metastases, old MI
09-92-89	86	m	160/90	LV	30 pack year smoking history old & recent MI,
43-81-00	77	f	140/62	LV	40 pack-year smoking history left middle cerebral artery infarction, bronchopneumonia
32-98-29	86	f	180/90	LV	acute hemorr. pancreatitis
37-75-23	83	m	170/90	LV	massive cerebral hemorrhage
52-25-64	25	f	130/80	NV	atypical sclerosis of white matter (?astrocytoma)
27-50-28	25	f	130/56	NV	lymphoma

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I.D.	Age	Sex	B.P.	Diet	Diagnosis
41-26-85	42	f	110/50	NV	acute myelogenous leukemia
14-20-14	75	f	150/90	NV	carcinosarcoma of uterus with metastases
36-18-24	70	f	120/70	NV	chronic active hepatitis & cirrhosis, diabetes(AODM)
02-77-46	81	f	200/95	LV	acute MI with ventricular rupture
12-04-03	94	f	120/70	LV	Cerebral infarct, old & recent
23-26-07	57	f	100/60	LV	Malignant lymphoma

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